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DESCRIPTION OF A SIMPLE DEVICE FOR RECORDING VARIATIONS IN
LUMINESCENT ABSORPTION DURING ULTRACENTRIFUGATION
FOR PURPOSES OF ANALYSIS

C. R. Acad. Sc. Paris,
Vol. 258, No. 24, 1964,
pages 5999-6002

Jean-Claude Deschepper and
Roland Van Rapenbusch and
presented by Francis Perrin

This device can be used on an analytical ultracentrifuge. A controlled logarithmic potentiometer recorder traces graphs of optical densities to wave lengths (ultraviolet and visible) selected by filters. Other than their numerical yield, these recordings show the immediate verification of the sedimentation phenomena.

In analysis by sedimentation, the refractometric and interferential methods, usually employed, cannot distinguish chemically the molecules; moreover, they use several milligrams of the substance under study.

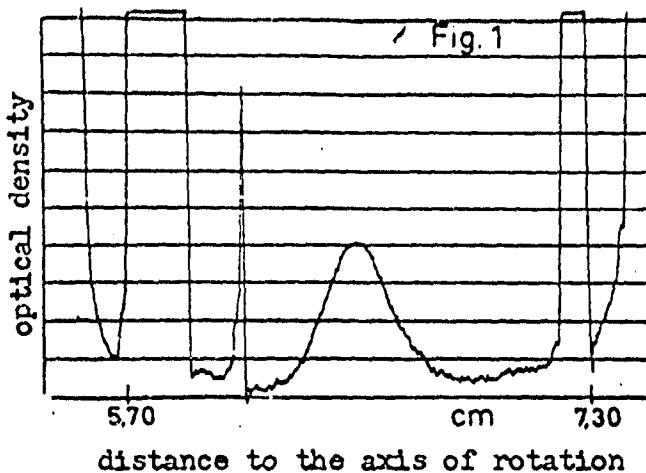
The specific and sensitive method of choice is light absorption which was first used by Svedberg (1), with photographic emulsion as the detector. The idea of replacing this with a photomultiplier tube seems to come from Schachman (2).

To date, three types of apparatus have been described (3-6). We offer different and simple equipment which we have built. This device produces graphs like that in Figure 1: the abscissas are proportional to the distances to the axis of rotation and the ordinates indicate the optical density in each point of the centrifuge tube.

This record (Fig. 1) represents a "band" (?) of human hemoglobin during sedimentation (wave length: 405 m μ ; total protein mass: 25 μ g).

Description -- The optical image of the sample is scanned automatically and at predetermined times, by a slot ($0.1 \times 7 \text{ mm}^2$) placed against the window of a photomultiplier tube. The latter transmits to

a logarithmic recorder a voltage proportional to the luminous intensity which it receives.



Our apparatus is adjustable on three axes at right angles to one another. It can use different light sources: xenon, mercury or deuterium tubes. A small slot (0.1 mm) delimits the light beam. The desired wave length is obtained by the interposition of a collection of optical filters.

The photomultiplier (RCA 1 P 28 or Radioteknique 150 UVP) is placed with a preamplifier in a metal unit integral with a carriage; the carriage is drawn at a speed of 4 mm/s, by a precision screw driven by a triphased synchronous motor running at 1000 rpm. The gear reduction comprises a system of pulleys and belts. All friction surfaces have ball bearings.

The recording apparatus (8) is a logarithmic servo-potentiometer. The signal is amplified then detected before being electrically filtered to eliminate stray frequencies due to rotor movement. A synchronous motor unrolls paper at 10 mm/s; the motor is fed by the same network that serves the scanning device. The variations in optical density are directly recorded on this paper. The effective time of transcription is eight seconds. The total enlargement G (produced from the optical enlargement by the recorder enlargement) is equal to 4X. Other values for G , larger or smaller can be chosen.

The writing speed is 2 m/s, which permits an actual resolution of 50 μ for an infinite slope transition. A better resolution, necessary for the analysis of thermodynamically balanced systems, can be obtained by another choice of scanning and recording speeds.

We take into consideration the lighting errors by using two centrifuge tubes mounted with prismatic windows: one contains solvent. The separation of images is performed optically by a system of our construction.

Operation -- Given I , an instantaneous value for the luminous intensity received by the photocathode; it is expressed on the anodic resistance of the photomultiplier charge by a negative impulse whose size is proportional to I and independent of the geometry of the tube as long as it has radial sides. The value of the coefficient of proportionality k depends on the voltage applied to the dynodes of the phototube and the rotation speed. These last parameters must remain fixed.

By construction, the recording stylus expresses the size kI of the impulse by a displacement, y . Since b is a constant, we have:

$$y = b \log (kI).$$

If the factor α represents the homologous values corresponding to the zone of the solvent, we get:

$$y_\alpha - y = b \log \left(\frac{I_\alpha}{I} \right) = b (\text{DO.}),$$

(DO.) designates the optical density of the solution at the spot under consideration.

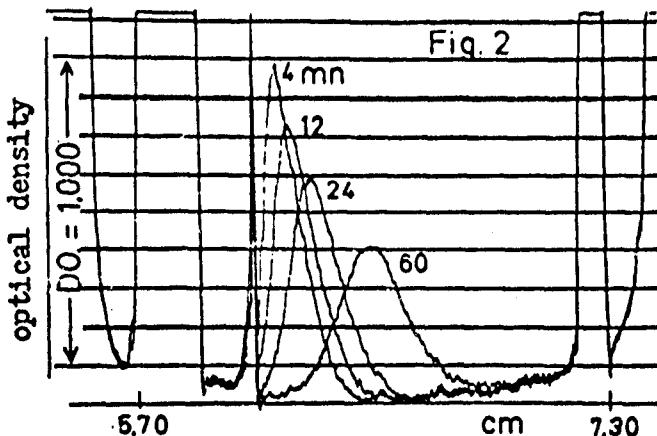
The coefficient b thus represents the sensitivity of the potentiometer in the operating mechanism and can be represented by cm/u.DO. (centimeter per unit of optical density). By an easy alteration of this potentiometer, we can use the following values of b : 2, 4 and 10 cm/u.DO.

Characteristics -- The precision of the measurement of the distance separating two transitions of the fixed face (for example, band of reference and air-solution miniscus) is 7 0/00; this is true no matter what the distance under consideration is. The enlargement of the radial distances is illustrated by Fig. 2; wherein are shown superimposed recordings of one hemoglobin "band" (?) obtained at different times (4, 12, 24 and 60 minutes after attaining the normal rate of 59780 rpm).

On the optical density axis, the scale is linear from zero to 1.30 u. DO. The mechanical pace of the potentiometers (a little less than 0.5 mm) limits the greatest precision to $\pm 1\%$. At present it is actually $\pm 4\%$.

Considered improvements -- A system with two beams is being assembled. It uses an auxiliary photodiode and two standard centrifuge tubes to bring the signal I_α/I to the input of the logarithmic recorder. This contrivance will mitigate the optical shortcomings and will show

weak differences in the coefficients of sedimentation and diffusion. In like manner, we hope to attenuate the residual imperfections in the recording apparatus.



NOTES

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Laboratory of Physical Biochemistry,
Institute of Research in Blood Diseases,
Saint Louis Hospital, Paris.